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FOR THE PERIOD

ELECTROPHORETIC SEPARATOR FOR PURIFYING BIOLOGICALS

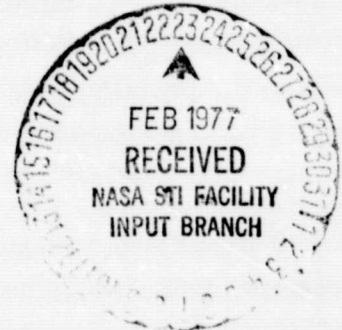
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## I. INTRODUCTION

One of the most promising ideas identified for space processing of materials is the separation and/or purification of biological materials by electrophoresis. Although electrophoresis was first observed in the early nineteenth century, its utilization as a laboratory technique did not take place until the 1930's when precise measurements were made on purified proteins and complex biological materials. Most of the development in electrophoresis since 1950 has been in an area described as zone electrophoresis in stabilized media. This technique separates a single narrow zone of sample mixture in an electrolyte medium into many zones containing a single component of the mixture and electrolyte between them. Since the densities of the separated zones generally differ from that of the intervening medium, such systems are gravitationally unstable and stabilization is required. The various techniques for stabilization include using the capillary spaces provided by thin films, the interstices of solid material such as filter paper and a variety of gel-forming substances.

Although zone electrophoresis has been adapted for both continuous and batch fractionation, much care and ingenuity have been involved in the choice and preparation of the stabilizing medium. This method is usually satisfactory for separating low molecular weight materials but it is impractical for high molecular weight materials because of immobility in the stabilizing media, and the interactions between the macroion and the stationary phase.

Attempts have been made to use fluid electrophoresis for the separation of high molecular weight materials which have little or no mobility in a porous solid. In the presence of gravity, however, the method is characterized by incomplete separations, due to electrophoretic and hydrodynamic effects near

the cell wall, and to density differences between the solvent and the solute. In addition, large-scale separations are limited by thermal convection arising from Joule heating of the solution.

Electrophoresis done in space will alleviate at least two major problems that occur on earth. (1) The electric field produces an electric current in the liquid medium which results in Joule heating. This heating generates convection currents in the solution which mix the components already separated. (2) Large biological particles of high density, such as living cells, settle to the bottom of liquid electrophoresis beds and cannot be effectively separated. Under weightless conditions, electrophoresis can be applied to molecules or particles of any size or density suspended in fluid media. The advantages are expected to make electrophoretic separation in space practical for preparing medical and biological products of high social and economic value.

The objectives of this project are:

- (1) to develop the analytical and experimental basis for a thick cell, free-flow electrophoresis separator for application in the low gravity of space;
- (2) to design, build, and test an engineering model of the electrophoretic separator;
- (3) to demonstrate the experiment operation on the ground with simulated weightless conditions;



- (4) to operate the model and conduct experimental simulation studies to provide data and experience that will predict the performance of the equipment in the space environment;
- (5) to predict the operation in space based upon analysis and experimentation and identify problem areas that remain unresolved by ground evaluation that must be tested or measured in space; and
- (6) to define flight experiments of varying duration that will demonstrate the performance of the design and apparatus concepts.

## II. STABILITY STUDIES AND MATH MODELING

### A) Stability Studies

In order to assess the real benefits to be gained from operating a thick electrophoresis cell in a micro-gravity we undertook a series of stability studies with a prototype ESE cell. Tests were performed to determine the relationship between residence time of the sample and the onset of thermal instability. The sample used was a neutral density latex to avoid buoyancy problems. The stability criterion was: a visibly stable sample stream for at least three minutes. The three minute limit was chosen to match the 'micro-g' period of a sounding rocket. A number of orientations were investigated from vertical ( $0^{\circ}$ ) to horizontal ( $90^{\circ}$ ). The results of these data are shown in Figure 1. It is interesting to note that the  $45^{\circ}$  orientation was most favorable and that the  $90^{\circ}$  orientation was only marginally better than the  $0^{\circ}$  orientation.

What this illustrates is that at reasonable residence times it is not possible to apply sufficient power to the thick cell in order to perform a useable separation.

Figure 1 was then combined with the curve which describes the power into the cell as a function of thickness (constant voltage gradient). This results in the surface shown in Figure 2. The dotted line indicates the intersection of the surface for the AAFE ESE cell. Points lying below the surface should be in regions of stable operation, those on or above the surface indicate a need to reduce gravity driven convection.

#### B. Math Modeling

In addition to the experimental data we used the math model results to graphically illustrate the advantage of a thick cell. We chose four mobility values similar to those of sheep, dog and two human erythrocytes (RBC). The cases we modeled were similar in cross sectional area to an Elphor, Beckman CPE and the AAFE ESE systems.

In each case the residence time at the centerline of the unit is the same, as is the sample stream diameter, voltage gradient and wall zeta potential. The results are shown in the following illustrations.

Figure 3 shows the results to be expected in a very thin electrophoresis cell such as any early Elphor system. Because the sample stream is so close to the walls the hydrodynamics of the fluid cause severe distortion of the sample bands. The residence time at the centerline is ca 300 seconds while at the edge of the sample stream it is ca 1700 seconds. As seen in the accompanying graph no significant amount of pure material is obtainable.

Figure 4 illustrates the kind of results to be expected (and we do see this) in an electrophoresis apparatus such as the Beckman CPE Mark II. Wall effects still predominate in this system and Beckman has taken elaborate steps to minimize these effects in the Mark II system. However, note that by making the electrophoresis chamber thicker (1.6 mm over 0.7 mm) it is now possible to obtain two of the four components in good purity at moderate (75%) to good (90%) yield.

Taking this one step farther, Figure 5 depicts the expected results for an AAFE-ESE system operating in micro-g. Both wall and hydrodynamic effects are at a minimum, purity of each sample would be excellent as would the yield of each.

On the recommendation of the COR, we checked our model against another model, more recently assembled at Lehigh University. We obtain a data set by phone and a copy of their printout by mail. We used their data set as inputs to our program, then compared our results with theirs. For the case which we examined, where potential of the wall nearly matched that of one of the sample particles, the differences in the calculations were minimal and the results compared nicely. In general, our model calculated slightly greater displacements ( $\sim .06$  cm or 8.5%) and slightly greater distortion (.01 cm). Upon receiving their printouts, we discovered two discrepancies between the data set given on the phone and the data they apparently used in their calculations. These were as follows: cell thickness was given to us as 0.15 cm, while they apparently used 0.1 cm; sample stream radius was given as 0.02 cm while they used an oval band 0.03 cm wide by 0.04 cm thick. Finally, we calculated a temperature gradient of  $1.1^{\circ}\text{C}$  while they assumed a gradient of  $5^{\circ}\text{C}$ . These differences



could account for the small disparity between the results. All in all, we are pleased with the results since this provides a useful check on our model.

### III. PROTOTYPE FLIGHT HARDWARE

#### A. Collection System

Early in this reporting period, we began to look at the changes necessary to allow collection of a biological material. The criteria were the collection of individual sample fractions and maintenance of sample sterility. These criteria required the modification and/or replacement of several system elements. Briefly, they are: modify old ESE cell, add multi-channel peristaltic pump, add removable collection container and modify old buffer pump system to be buffer/electrolyte storage.

The first step was to add the multi-channel collector to the ESE cell. This consists of fifty (50) stainless steel tubes .040" O.D. spanning 50 mm (2") of the cell width. The tubes are potted in epoxy and the assembly is mounted to one faceplate. The addition of the collector allowed the old ESE cell to be shortened about 2" and the cell could then be placed off axis to provide room for the pump and collection container.

The peristaltic pump is the prime mover in the ESE MOD II. It provides the driving force not only for buffer flow but also for electrolyte circulation. In order to provide a reproducible flow rate through each collector tube, the pump is on the outlet side of the electrophoresis cell and has fifty-two (52) channels, fifty for buffer and two for electrode electrolyte. The pump occupies an envelope 14 cm x 10 cm x 7.5 cm and weighs about 1.2 kg. The flow rate can be programmed so that the residence time in the cell can be set from about fifty (50) seconds to two-hundred (200) seconds.



After passing through the pump the sample fractions are collected in fifty (50) individual collection bags in a single prefrozen housing. This sample collection container is removeable through the use of a unique fifty-channel fluid connector based on an electrical connector. The male side of the connector is connected to the peristaltic pump and consists of fifty (50) 21g stainless steel needles. The female side of the connector remains with the collection container and has a silicone rubber septum to maintain sterility of the sample at removal.

The original buffer pump has been modified for buffer/electrolyte storage. The motor and piston were removed and use was made of the refrigerant in the annulus for keeping the stored fluids cold. The buffer and electrolyte are contained in bags inside the storage chamber. Access to the bags is provided by flow switching valves which allow the bags to be filled from an external source and then be connected to the ESE system. An additional bag is provided for electrolyte waste during operation. The valves are arranged so that the collection container will not enter and engage its mounting brackets if they are incorrectly positioned.

#### B. U.V. Detector System

In order to install the collection system on the MOD II ESE, it was necessary to remove the automated camera system to make room for the collection container. However, it was still desirable to have some way of recording the separation as it takes place. We began by bench testing a UV sensing optical system which employs a solid-state diode array as the sensor.

Preliminary tests with this linear diode array indicated that it would have sufficient sensitivity in the UV region to detect the biological materials of interest. Our first problem however, was to find a suitable power source

for the UV lamp. As purchased, the lamp operates from a 60 Hz supply. This was unacceptable since the array "saw" the 60 cycle modulation of the lamp. A DC supply was deemed impractical since this would ultimately cause the lamp to fail due to deposition of the mercury vapor at one of the electrodes and required on the order of 1000 volts. Therefore, we designed a DC to AC supply operating at 10 KHz. At this frequency there is no appreciable modulation of the sensor output; it "sees" a constant signal. We had been aware of the problem of overheating of the lamp, and consequently an increase in its output, through our own work and the ASTP results. Our lamp supply, therefore, incorporates a feedback sensor mounted near the lamp to keep its output constant once set.

Our next task was to design an optical system to allow the sensor to view the sample area just above the collection tubes. Several simple lens systems were tried on an optical bench, none of which proved satisfactory. Finally a three lens design was employed with good results and incorporated into our final design. The system works in this manner: the lamp is focused into the center of the cell while the array is arranged to look at the sample area from the other side. With no sample present, the lamp output is adjusted to give the maximum output signal. When sample passes by the viewing area, it will absorb some of the radiation and this will show up as a decrease in the signal at the array output. A 253.7 nm interference filter is used to block out unwanted wavelengths.

### C. Miscellaneous Hardware

We have made two other additions to improve the performance of the ESE MOD II. They are: modified cell face plates for improved thermal conductivity and stirrer mechanism in the sample pump.

Considering the possibility that the ESE might be required to separate a biological specimen of low mobility in a medium of relatively high ionic strength, and to collect some amount of separated material during a five minute rocket flight, we became concerned about heat transfer through the glass/epoxy (GRP) faceplates. We decided to try to improve the heat transfer characteristics of the chamber walls. Our first attempt was to use an aluminum faceplate to which a sheet of 0.002" glass was bonded. The advantage of the glass lining is that several good coatings exist which can be used to alter the zeta potential of a glass surface. We experienced some difficulties with leakage currents and decided to use an anodized aluminum impregnated with PTFE everywhere but under the glass. This coating, advertised to withstand 2000 volts, failed our 500 volt test. It became apparent that we needed high thermal conductivity only in the area of the cooling jacket (electrophoresis area) and we designed a composite faceplate of GRP and ceramic. The GRP faceplate was machined out to accept a piece of Alsimag 14, an alumina ceramic with a thermal conductivity of  $22 \text{ Btu} \cdot \text{ft}^2 \cdot \text{hr}^{-1} \cdot ^\circ\text{F}^{-1} \cdot \text{ft}^{-1}$  (about the same as stainless steel). Due to the increase in conductivity and a thinner plate the composite has a conductance about 300 times greater than plain GRP.

Our major concern was whether or not the Alsimag ceramic would behave like the glass surface when coated with the zeta-potential coatings. Our experiments indicate that we were successful in coating this ceramic.



Through our own research (not on this contract) and personal communications with others, we were made aware of a potential problem in the sample pump. Experiments performed with mammalian cells (e.g. lymphocytes) showed that these cells had a tendency to settle out of suspension while sitting. Therefore, a stirring mechanism was added to the sample pump. A set of field coils was installed inside the cooling jacket and a magnetic stirring bar was placed inside the syringe barrel. The field coils are pulsed by a 4-phase power supply constructed from 555 timers. This supplies a stirring rate of 1 revolution per second.

This set-up kept red blood cells in suspension for over two hours while an unstirred sample settled out.



Figure 1

POWER VS RESIDENCE TIME: 3 MINUTE STABILITY STANDARD  
NEUTRAL DENSITY LATEX

ANGLE IS MEASURED FROM VERTICAL

A

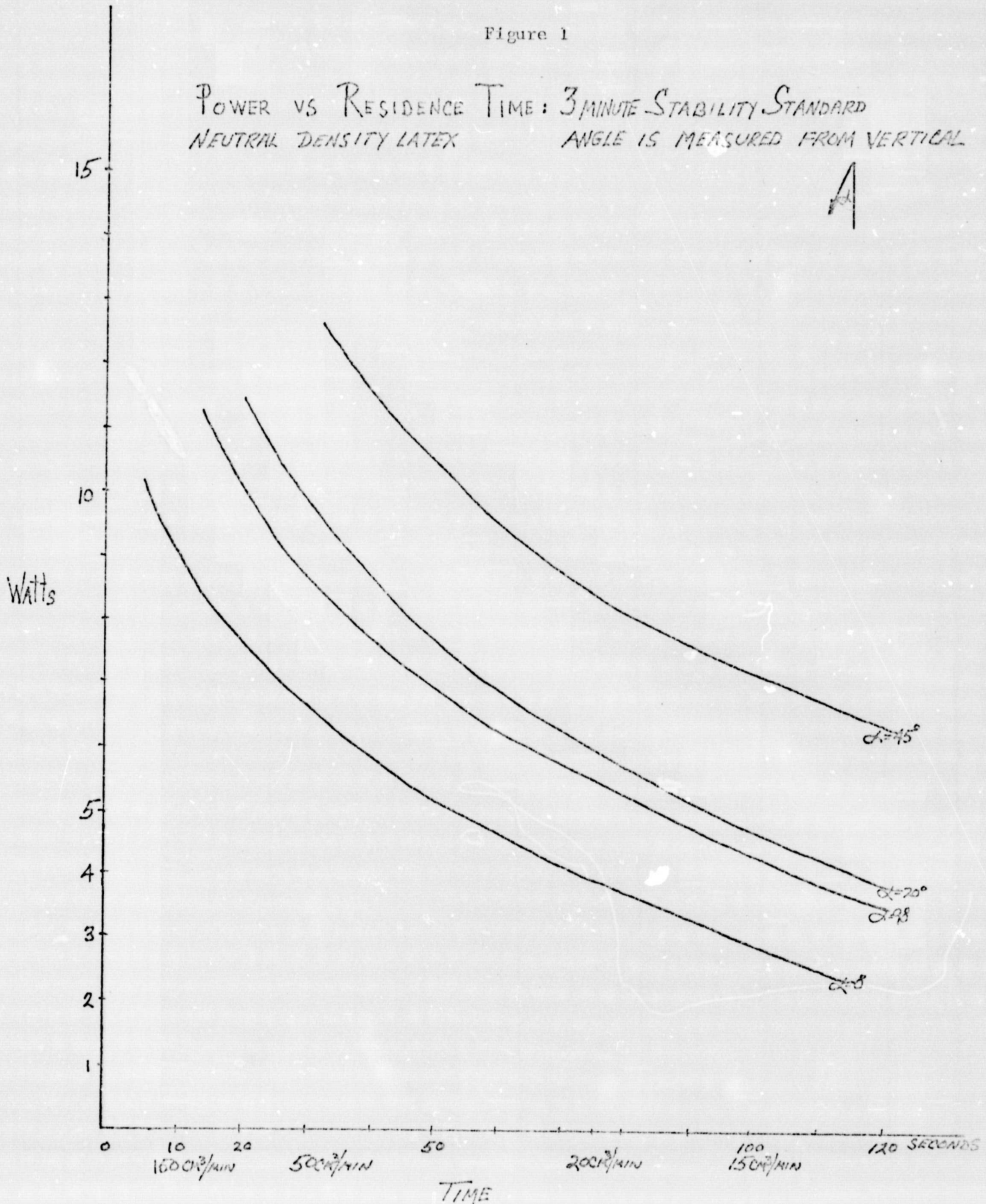
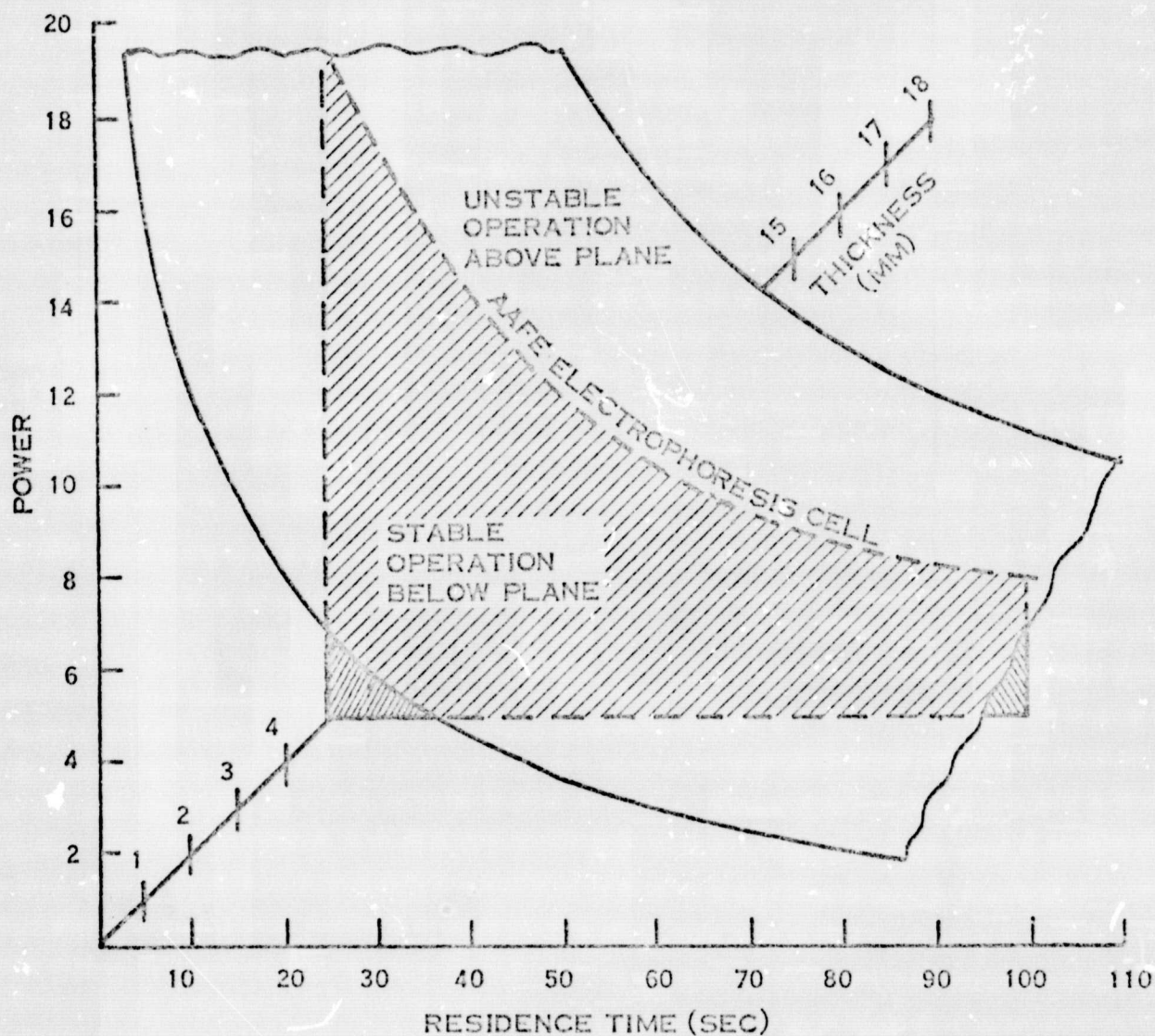
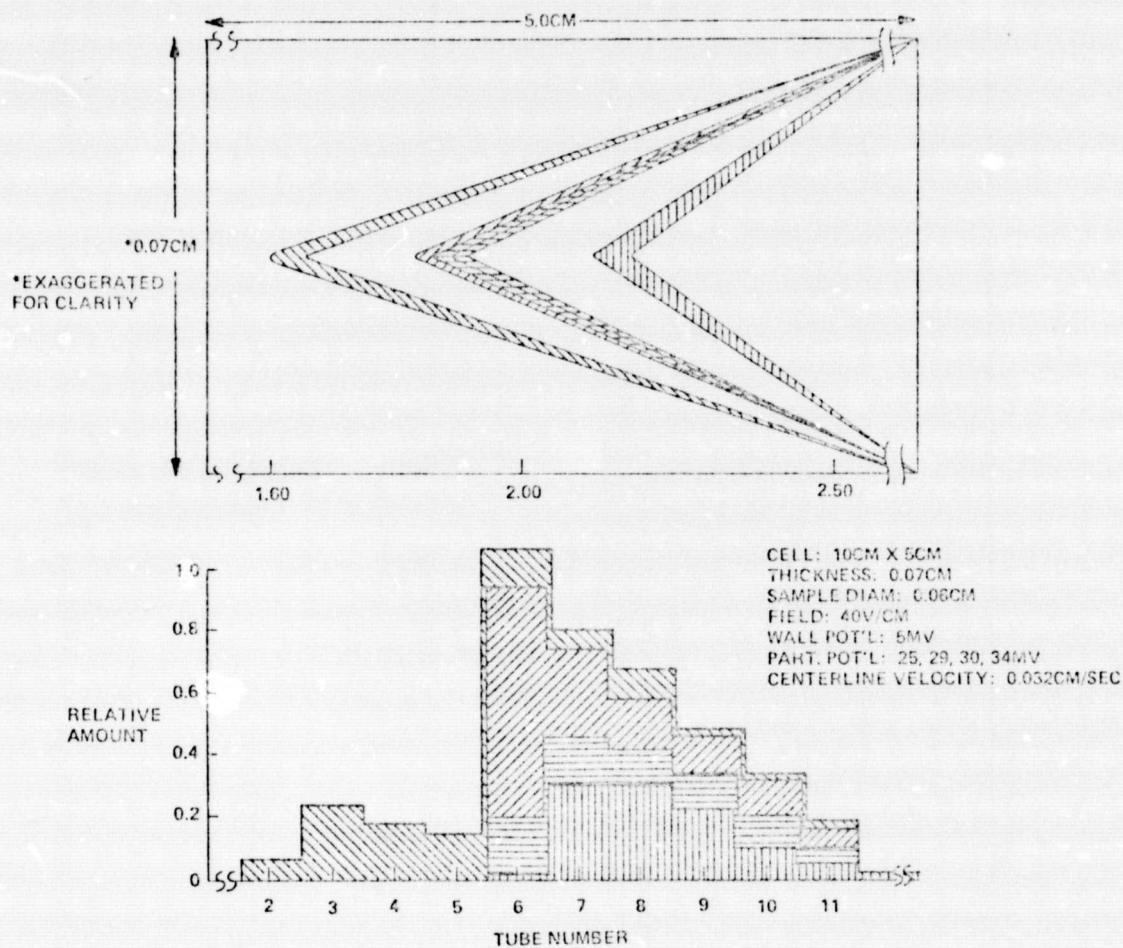


Figure 2 Experimentally determined regions of stable and unstable operation of an electrophoresis cell as a function of the power (in Watts) and the residence time for a 0.50 cm thick cell with other conditions estimated.



ACTIVE AREA 5CM X 10CM  
 SAMPLE: NEUTRAL DENSITY POLYSTYRENE LATEX  
 ORIENTATION 20° FROM VERTICAL

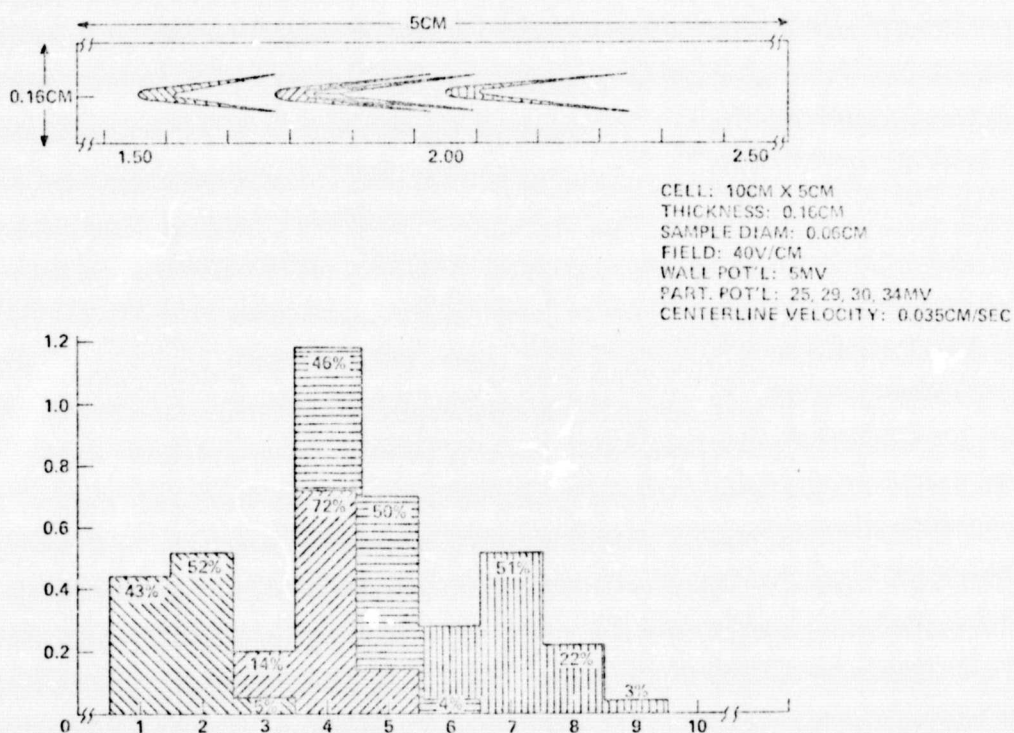
Figure 3



Graphic illustration of separation and resolution for a four component mixture in a 0.07 cm thick cell. Other conditions as indicated.



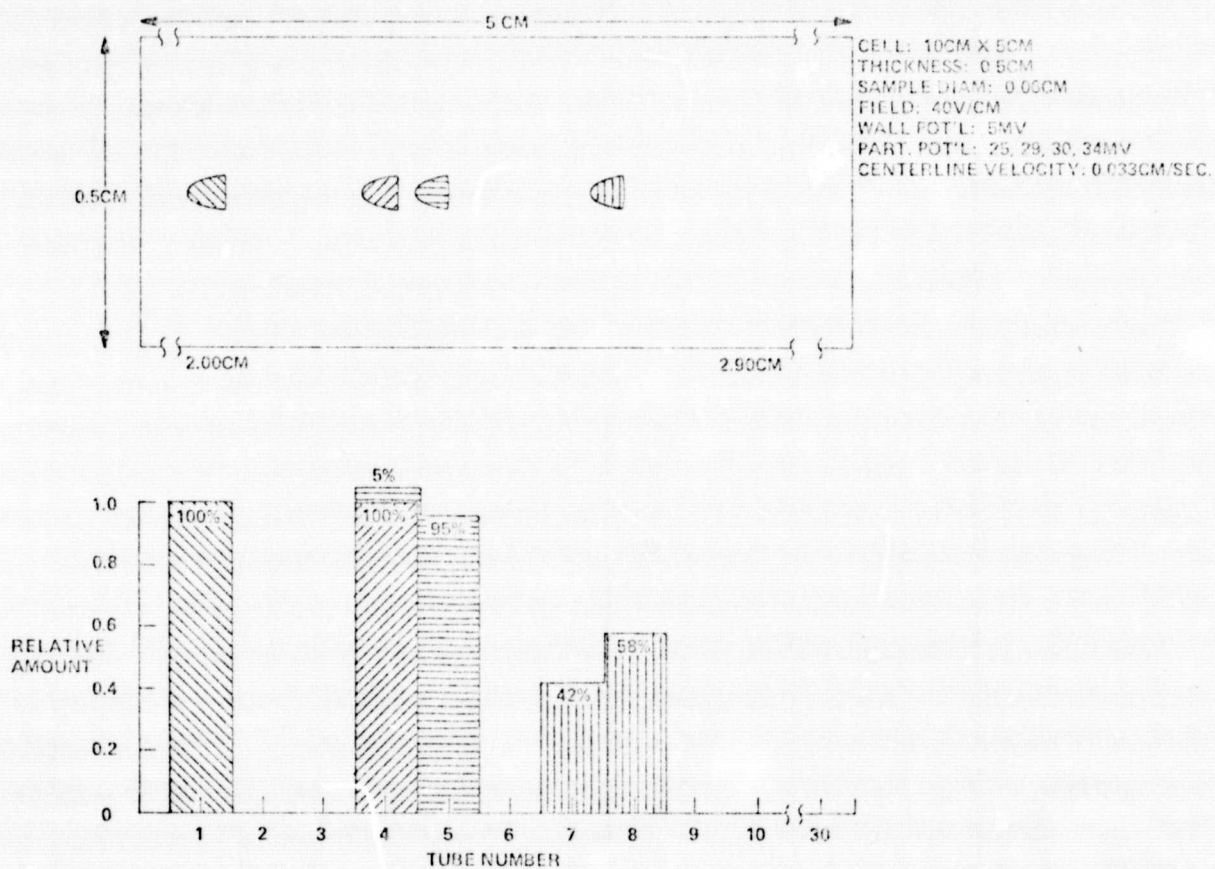
Figure 4



Graphic illustration of separation and resolution for a four component mixture in a 0.16 cm thick cell. Other conditions as indicated.



Figure 5



Graphic Illustration of Separation and Resolution for a four component mixture in a 0.5 cm thick cell.